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Fine-tuning of hematopoietic stem cell homeostasis: novel role for ubiquitin ligase

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Homeostasis of hematopoietic stem cells (HSCs) is a tightly regulated process, controlled by intrinsic and extrinsic signals. Although a variety of molecules involved in HSC maintenance and self-renewal are known, it remains unclear how robust HSC homeostasis is achieved. In this issue of *Genes & Development*, Rathinam and colleagues (pp. 992–997) report a new player in HSC homeostasis, c-Cbl ubiquitin ligase. They show that this E3 ubiquitin ligase acts as a negative regulator of cytokine signaling.

The E3 ubiquitin ligase c-Cbl is a member of the RING finger-type ubiquitin ligase Cbl (casitas B-cell lymphoma) family. It has been shown to interact with several tyrosine kinase receptors and other adaptor proteins to “fine-tune” lymphocyte antigen receptor signaling, mainly through ubiquitination (for review, see Duan et al. 2004). To study the role of c-Cbl in hematopoietic stem cells (HSCs), Rathinam et al. (2008) examined mice lacking *c-Cbl*. Surprisingly, they observed that the number of HSCs (CD34⁺Flt3⁺LSK or CD150⁺CD48⁺ cells) was increased in the bone marrow of c-Cbl-deficient mice, and that c-Cbl-deficient HSCs were more competent than wild-type HSCs in reconstituting the hematopoietic system. Examination of another c-Cbl mutant mouse line, in which E3 ligase activity was ablated but the tyrosine kinase-binding domain was preserved, showed an almost identical HSC phenotype to that of c-Cbl-deficient HSCs. These results strongly suggest that ubiquitin-mediated protein degradation is important for HSC homeostasis.

What is the target substrate for c-Cbl in HSCs? Rathinam et al. (2008) observed accumulation of signal transducer and activator of transcription 5 (STAT5) and phosphorylated STAT5 (activated form) in c-Cbl-deficient HSCs, and proposed that STAT5 is a candidate target for ubiquitin-mediated degradation. STAT5 is an intracellular transducer of several hematopoietic-specific

cytokine signaling pathways. Therefore, although there are many targets of c-Cbl (Duan et al. 2004), it is likely that STAT5 is a major target of c-Cbl in the context of HSC homeostasis.

HSC homeostasis and cytokine signaling

Many cytokines have been tested for their ability to stimulate self-renewal and expansion of HSCs in vitro. Thrombopoietin (TPO) and Stem Cell Factor (SCF) are recognized as important positive regulators of HSC self-renewal and expansion, although expansion has not been achieved thus far. The receptors for TPO and SCF—c-Mpl and c-Kit, respectively—are expressed on transplantable HSCs (Ikuta and Weissman 1992; Solar et al. 1998; Adolfsson et al. 2001; Yoshihara et al. 2007), and mice with genetic mutations in TPO, SCF, c-Mpl, or c-Kit are reduced in HSC numbers (Russell 1979; Kimura et al. 1998; Qian et al. 2007). Interestingly, Rathinam et al. (2008) show that c-Cbl-deficient HSCs are hypersensitive to TPO. TPO-c-Mpl activates three downstream signaling pathways: mitogen-activated protein kinase (MAPK) (Rojnuckarin et al. 1999), phosphoinositide 3-kinase-Akt (PI3K-AKT) (Geddis et al. 2001), and Janus kinase (JAK)–STAT3 and STAT5 (Dorsch et al. 1995; Drachman et al. 1995). Kato et al. (2005) reported that STAT5 activation was observed in HSCs (CD34⁺KSL cells) after TPO stimulation, and overexpression of STAT5 in HSCs enhanced their self-renewal activity *ex vivo*. Thus, it is likely that JAK–STAT is an important downstream pathway of TPO signaling for HSC maintenance. Consistent with this notion, HSCs lacking Lnk, an adaptor protein functioning as a negative regulator of several cytokine signaling pathways, show increased self-renewal capacity and an increased amount of phosphorylated STAT5 in the presence of TPO signaling (Ema et al. 2005; Buza-Vidas et al. 2006; Seita et al. 2007). The phenotypes of c-Cbl-deficient HSCs resemble those of Lnk-deficient HSCs, showing both hypersensitivity to TPO and increased phosphorylated STAT5 (Rathinam et al. 2008). Although it is uncertain whether there is a direct relationship between Lnk and c-Cbl, Rathinam et al. (2008) clearly show that c-Cbl modulates the c-Mpl–TPO–JAK–STAT signaling pathway in HSCs.

[**Keywords:** Hematopoietic stem cells; c-Cbl; thrombopoietin; STAT5; self-renewal]

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HSC maintenance in the bone marrow niche

HSC self-renewal and differentiation are thought to be controlled through interactions with the bone marrow niche (for review, see Wilson and Trumpp 2006). In the bone marrow, HSCs are associated with osteoblasts, endothelial cells, or CXCL12⁺ reticular cells (Calvi et al. 2003; Zhang et al. 2003; Kiel et al. 2005; Sugiyama et al. 2006). Although it is still unclear why several distinct niches exist in the bone marrow, various molecules have been proposed to be involved in the signaling between HSCs and the niche (Wilson and Trumpp 2006). Among them, Angiopoietin-1, a ligand for the Tie-2 receptor, is expressed by osteoblasts in the bone marrow niche and is important for maintaining HSC quiescence (Arai et al. 2004). Notch signaling also has been proposed to be crucial for HSC expansion in the niche, since Notch1 and Jagged1 are expressed by HSCs and osteoblasts, respectively (Calvi et al. 2003), and inhibition of Notch signaling by a dominant-negative protein results in accelerated differentiation of HSCs in vitro and depletion of HSCs in vivo (Duncan et al. 2005). However, conditional deletion studies reveal that these molecules are dispensable for HSC maintenance in vivo (Mancini et al. 2005).

Until recently, it was unclear whether TPO signaling is involved in HSC maintenance within the bone marrow niche. However, Yoshihara et al. (2007) and Qian et al. (2007) reported that TPO is expressed by osteoblasts in the bone marrow niche and is involved in HSC quiescence and expansion. Given that c-Cbl-deficient mice have increased numbers of HSCs in the bone marrow and c-Cbl-deficient HSCs are hypersensitive to TPO (Rathinam et al. 2008), it is plausible that c-Cbl modulates the signal from TPO-producing osteoblasts in the bone marrow niche through the ubiquitination of STAT5. Of note, the JAK-STAT signaling pathway is also involved in blood cell homeostasis in *Drosophila*. Like the mouse bone marrow niche, a small cluster of cells in the primary lobe of the *Drosophila* lymph gland—termed the posterior signaling center—controls the balance between multipotent prohemocytes and differentiating hemocytes. In STAT mutant flies, increased hemocyte differentiation has been observed (Krzemien et al. 2007), implicating JAK-STAT signaling in the maintenance of undifferentiated hemocyte progenitors. Thus, the JAK-STAT signaling pathway may be evolutionally conserved in function, providing maintenance of stem cells within the niche.

While accumulating evidence indicates that interaction between stem cells and a niche is critical for stem cell maintenance, two recent studies have demonstrated that there is a competition between stem cells for niche occupancy. Jin et al. (2008) demonstrated that in *Drosophila*, *bgen* and *bam* mutant germline stem cells (GSCs) push wild-type GSCs out of the niche because of their higher expression of E-cadherin. Similar stem cell competition has been observed in the mouse bone marrow niche. Czechowicz et al. (2008) showed that treatment with ACK2, an antibody that blocks c-Kit function, cleared host HSCs from the niche and enabled HSC en-

graftment without irradiation. In addition, the number of HSCs in the bone marrow has been shown to correlate with the number of osteoblasts (Calvi et al. 2003). These results strongly suggest that the number of niches is limited. If so, where within the bone marrow environment are the surplus HSCs in c-Cbl-deficient mice localized? Or, in the steady-state bone marrow, are the niches not fully occupied by HSCs? One possibility is that c-Cbl-deficient HSCs have a greater adhesive ability for the niches than wild-type HSCs and associate with “incomplete niches,” thus resulting in higher numbers of HSCs in the bone marrow. Consistently, Rathinam et al. (2008) observe that c-Cbl-deficient HSCs are more competent than wild-type HSCs in bone marrow reconstitution. Further detailed and quantitative histological analysis of the bone marrow of c-Cbl-deficient mice may answer this question.

Outside the bone marrow: HSC homeostasis in embryos

During embryonic stages, HSCs are generated prior to the establishment of the bone marrow niches. It is still unclear whether the embryonic niches that maintain HSCs are equivalent to adult bone marrow niches. Stromal cell lines isolated from several embryonic HSC niches (aorta-gonad-mesonephros region and liver) have been shown to support the maintenance of adult bone marrow HSCs (Oostendorp et al. 2005; Durand et al. 2007). However, other studies indicate that the properties of HSCs differ between embryonic and adult stages, although it seems that they share the same embryonic origin (Göthert et al. 2005; Samokhvalov et al. 2007).

HSCs change from a rapidly proliferating state to a quiescent state between 3 and 4 wk after birth (Bowie et al. 2006). Moreover, conditional depletion of the Sox17 transcription factor revealed that it is required for the maintenance of fetal and neonatal HSCs, but not adult HSCs (Kim et al. 2007). In contrast, Tie receptors are important for regulation of adult HSCs, but not fetal HSCs (Puri and Bernstein 2003). Sensitivity to cytokines is also different between HSCs from embryonic and adult stages. Fetal HSCs are more sensitive to SCF than adult HSCs despite the same expression level of c-Kit (Bowie et al. 2007). In addition, TPO is crucial for expansion of adult HSCs, but not fetal HSCs (Qian et al. 2007). To date, it is unknown what factors modulate the sensitivity to these cytokines. It is now worth considering the possibility that protein modifications like ubiquitination affect the sensitivity of HSCs to cytokines.

Does aberrant control of ubiquitination lead to leukemia?

It has been well documented that regulators of HSC maintenance are also involved in leukemic induction (for review, see Rizo et al. 2006). Although Rathinam et al. (2008) have not observed tumor formation in c-Cbl-

deficient mice, it is possible that accumulation of STAT5 in c-Cbl-deficient HSCs increases susceptibility for leukemic transformation, since overexpression of STAT5 in HSCs induces myeloproliferative disease (Kato et al. 2005). In this regard, the study by Rathinam et al. (2008) might be extrapolated to other molecules like PTEN, TEL, and Gfi1, which have been reported to be involved in both HSC homeostasis and leukemia (Akagi et al. 2004; Hock et al. 2004a,b; Yilmaz et al. 2006; O'Neil and Look 2007). These proteins are also ubiquitinated (Chakrabarti et al. 1999; Marteiijn et al. 2007; Trotman et al. 2007; Wang et al. 2007). Thus, dysregulation of ubiquitin-mediated degradation may result in HSC exhaustion or tumor formation. Indeed, it has been reported that the PTEN protein level controlled by NEDD4-1 ubiquitin ligase influences its tumor suppressor activity (Trotman et al. 2007; Wang et al. 2007).

The study by Rathinam et al. (2008) now opens a new avenue for understanding the regulation of intracellular signaling and a further mechanism underlying HSC homeostasis. While researchers have looked previously to surface signaling molecules on HSCs to prospectively isolate and characterize HSCs, we should now begin to focus on the fine-tuning of intracellular signaling molecules by ubiquitination events inside HSCs. With the improvement of detection methods, it will be important to demonstrate the direct targets of ubiquitination in HSCs.

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